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(54) Title: HUMAN GENES AND GENE EXPRESSION PRODUCTS II

(57) Abstract

This invention relates to novel human polynucleotides and variants thereof, their encoded polypeptides and variants thereof, to genes corresponding to these polynucleotides and to proteins expressed by the genes. The invention also relates to diagnostic and therapeutic agents employing such novel human polynucleotides, their corresponding genes or gene products, e.g., these genes and proteins, including probes, antisense constructs, and antibodies.

having the sequence of one or more polynucleotides of the invention can be synthesized. Thus, the invention encompasses nucleic acid molecules ranging in length from 15 nucleotides (corresponding to at least 15 contiguous nucleotides of one of "SEQ ID NOS : 1-5252") up to a maximum length suitable for one or more biological manipulations, including replication and expression, of the nucleic acid molecule. The invention includes but is not limited to (a) nucleic acid having the size of a full gene, and comprising at least one of "SEQ ID NOS : 1-5252" ; (b) the nucleic acid of (a) also comprising at least one additional gene, operably linked to permit expression of a fusion protein ; (c) an expression vector comprising (a) or (b) ; (d) a plasmid comprising (a) or (b) ; and (e) a recombinant viral particle comprising (a) or (b). Once provided with the polynucleotides disclosed herein, construction or preparation of (a)- (e) are well within the skill in the art.

The sequence of a nucleic acid comprising at least 15 contiguous nucleotides of at least any one of "SEQ ID NOS : 1-5252", preferably the entire sequence of at least any one of "SEQ ID NOS : 1-5252", is not limited and can be any sequence of A, T, G, and/or C (for DNA) and A, U, G, and/or C (for RNA) or modified bases thereof, including inosine and pseudouridine. The choice of sequence will depend on the desired function and can be dictated by coding regions desired, the intron-like regions desired, and the regulatory regions desired. Where the entire sequence of any one of "SEQ ID NOS : 1-5252" is within the nucleic acid, the nucleic acid obtained is referred to herein as a polynucleotide comprising the sequence of any one of "SEQ ID NOS : 1-5252." II. Expression of Polypeptide Encoded by Full-Length cDNA or Full-Length Gene The provided polynucleotide (e. g., a polynucleotide having a sequence of one of "SEQ ID NOS : 1-5252"), the corresponding cDNA, or the full-length gene is used to express a partial or complete gene product. Constructs of polynucleotides having sequences of "SEQ ID NOS : 1-5252" can be generated synthetically. Alternatively, single- step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides is described by, e. g., Stemmer et al., *Gene* (Amsterdam) (1995) 164 (1) : 49-53. In this method, assembly PCR (the synthesis of long DNA sequences from large numbers of oligodeoxyribonucleotides (oligos)) is described. The method is derived from DNA shuffling (Stemmer, *Nature* (1994) 370 : 389-391), and does not rely on DNA ligase, but instead relies on DNA polymerase to build increasingly longer DNA fragments during the assembly process.

Appropriate polynucleotide constructs are purified using standard recombinant DNA techniques as described in, for example, Sambrook et al., *Molecular Cloning : A Laboratory Manual*, 2nd Ed., (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY, and under current regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. The gene product encoded by a polynucleotide of the invention is expressed in any expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. Suitable vectors and host cells are described in U. S. Patent No. 5, 654, 173.

Bacteria. Expression systems in bacteria include those described in Chang et al., *Nature* (1978) 275 : 615 ; Goeddel et al., *Nature* (1979) 281 : 544 ; Goeddel et al., *Nucleic Acids Res.* (1980) 8 : 4057 ; EP 0 036, 776 ; U. S. Patent No. 4, 551, 433 ; DeBoer et al., *Proc.*

Any of a variety of detectable labels can be used in connection with the various embodiments of the diagnostic methods of the invention. Suitable detectable labels include fluorochromes, (e. g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2', 7'-dimethoxy-4', 5'- dichloro-6-carboxyfluorescein, 6-carboxy-X-rhodamine (ROX), 6-carboxy-2', 4', 7', 4, 7- hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N, N, N', N'-tetramethyl-6- carboxyrhodamine (TAMRA)), radioactive labels, (e. g. 32p, 35S, 3H, etc.), and the like. The detectable label can involve a two stage systems (e. g., biotin-avidin, hapten-anti-hapten antibody, etc.) Reagents specific for the polynucleotides and polypeptides of the invention, such as antibodies and nucleotide probes, can be supplied in a kit for detecting the presence of an expression product in a biological sample. The kit can also contain buffers or labeling components, as well as instructions for using the reagents to detect and quantify expression products in the biological sample. Exemplary embodiments of the diagnostic methods of the invention are described below in more detail.

Pharmaceutical Compositions and Therapeutic Uses Pharmaceutical compositions can comprise polypeptides, antibodies, or polynucleotides of the claimed invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation is determined by routine experimentation and is within the judgment of the clinician. For purposes of the present invention, an effective dose will generally be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N. J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.